

FREE AMINO ACIDS IN CEREBROSPINAL FLUID*

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Values for total amino acid nitrogen and for certain individual amino acids in cerebrospinal fluid have been reported in the literature. The values for total α -amino acid nitrogen show some variations, perhaps depending on the methods employed. Weichmann and Dominicke (1), using the Folin colorimetric method, report values of 1.2 to 2.0 mg. per cent, while Christensen *et al.* (2), using the manometric ninhydrin procedure, report 1.04 to 1.43 mg. per cent of amino nitrogen. Harris (3) has reported the glutamine content of cerebrospinal fluid to be 6.0 to 11.9 mg. per cent. Christensen *et al.* (2), using chemical methods, report the glycine values for spinal fluid to be about one-tenth and the alanine values to be about one-third of the corresponding values for blood plasma.

We are presenting results of the determination by microbiological methods of eleven individual amino acids in spinal fluid.

EXPERIMENTAL

The microbiological determination of amino acids in spinal fluid offered some difficulty because of the small amounts of the amino acids present and because of the occurrence in spinal fluid of substances interfering with growth of the test organisms. An attempt to run estimations on spinal fluid without protein removal was unsuccessful because of growth inhibition. The use of tungstic acid as an agent for protein removal was not satisfactory. One difficulty lay in removing the protein completely without leaving an excess of tungstic acid in the filtrate.

We next tried heating spinal fluid at pH 4.7 to coagulate the protein and then filtering. Removal of protein was fairly complete but inhibition remained. We then evaporated this filtrate for 3 hours on a boiling water bath and found that the resulting solution could be successfully analyzed. The nature of the inhibiting substances destroyed by this treatment is not known. Our final procedure gave satisfactory recoveries of amino acids added to spinal fluid in amounts similar to those already present in the fluids. There were no significant changes in the α -amino nitrogen values of

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spinal fluid treated according to our procedure, indicating that there was no hydrolytic liberation of amino acids involved.

Procedure

The spinal fluid specimens analyzed were obtained from patients undergoing encephalography at the Illinois Neuropsychiatric Institute. Some of the patients were epileptic. All represented conditions in which no abnormality of the spinal fluid has been reported. There were no cases of syphilis, meningitis, or other inflammatory conditions. All specimens were clear and free from blood. The volumes varied usually from 50 to 100 cc., with some smaller specimens from children. The spinal fluids, collected by lumbar puncture under sterile conditions, were kept in a frozen state until analyzed. From 40 to 80 cc. of the spinal fluid were transferred to a 150 cc. beaker. The pH was adjusted with hydrochloric acid to 4.7 and the fluid then heated for 30 minutes on a boiling water bath. Coagulated protein was removed by filtration, the precipitate washed twice with 4 to 5 cc. of distilled water, and the filtrate returned to a beaker on the boiling water bath for about 2 hours longer. The fluids were thus concentrated to about one-fourth the original volume. The pH was adjusted to 6.8 with 0.1 N sodium hydroxide and diluted to a definite volume (one-fourth of the original volume).

The concentrated fluid was analyzed microbiologically for amino acids according to the procedures previously outlined for blood plasma and sweat (4, 5).

RESULTS AND DISCUSSION

Table I gives the analyses of fluids from sixteen non-epileptic subjects. It will be seen that there are no great variations in the amount of the individual amino acids in the fluids from the various subjects.

Table II presents values for fluids from patients with epilepsy. It will be seen that the results for the individual amino acids fall in about the same range as the values in Table I (non-epileptic group). Means and standard deviations are given for the entire twenty-six spinal fluids. In Table II are also included values obtained on two composite samples of spinal fluid, one from adults (Group A (5), ages 45 to 62 years) and the other from children (Group B (5), ages 2 to 12 years). It will be seen that the values for the older age group are similar to those in Tables I and II, which included chiefly individuals from 25 to 60 years of age. The lower age group shows generally lower values for the individual amino acids. Further analyses in this lower age group are desirable. Children have been reported to show lower values than adults for blood amino acid nitrogen (6).

In Table III a comparison is made of the levels of free amino acids in

spinal fluid with those in human plasma (4). The ratios of plasma values for spinal fluid are much lower, ranging from one-fifteenth to one-fourth of the values for plasma, with an average of about one-tenth. Arginine, with a ratio of 4, is somewhat exceptional. This somewhat independent position

TABLE I

Free Amino Acid Content of Cerebrospinal Fluid from Sixteen Non-Epileptic Subjects

Reported as micrograms of amino acid per cc. of cerebrospinal fluid.

Specimen No.	Diagnosis	Age	Arginine	Histidine	Isoleucine	Leucine	Lysine	Phenylalanine	Threonine	Tyrosine	Valine	Methionine	Cystine
			yrs.										
1	Cortical atrophy	57 ♂	4.8	1.8	0.45	1.0	1.5	1.6	3.6	1.9	1.9		
2	Porencephaly	25 ♂	3.9	1.8	1.1	1.4	0.66	0.82	1.8	0.55	2.5		
3	Encephalopathy	27 ♂	6.4	1.5	1.6	1.0	2.4	1.8	1.8	2.1	1.9		
4	Tuberous sclerosis	26 ♂	7.7	2.0	2.7	1.3	2.0	2.0	3.1	1.6	2.6		
5	Encephalopathy	60 ♂	5.8	1.7	1.5	1.8	2.9	2.6	3.2	2.7	2.8		
6	Hemiatetosis	36 ♂	6.8	2.0	1.0	1.2	2.6	1.7	2.5	1.9	2.5		
7	Encephalopathy	30 ♂	7.4	1.8	1.3	1.4	2.8	2.7	2.3	2.4	2.3		
8	Cortical atrophy	34 ♀	3.5	1.4	0.78	0.95	3.5	1.6	2.3	1.9	1.6		1.6
9	" "	42 ♀	3.7	1.6	0.71	1.4	3.2	1.7	1.9	2.0	1.6		1.7
10	Chorea, chronic	26 ♂	5.5	2.0	0.60	1.7	4.0	2.4	2.9	2.2	2.7	0.40	1.6
11	Cerebral throm- bosis	38 ♂	6.5	1.4	0.50	1.3	3.5	2.5	2.5	2.4	1.6	0.43	2.7
12	Encephalitis lethargica	38 ♂	6.3	1.8	0.65	1.5	3.6	1.9	3.8	2.3	2.2	0.45	1.4
13	Cortical atrophy	28 ♂	5.7	1.4	0.65	1.6	3.4	1.7	3.7	1.6	2.2	0.28	1.6
14	Cerebral degenera- tive disease	52 ♀	6.7	1.5	0.60	1.4	3.0	2.3	2.7	2.0	2.1	0.38	2.7
15	Dilatation of cavum	25 ♀	6.0	1.9	0.75	1.7	3.9	1.4	3.9	1.9	2.5	0.43	0.85
16	Convulsive state	44 ♂	4.8	1.4	1.9	1.3	2.9		2.9	1.9	1.9		
	Mean.....		5.7	1.7	1.04	1.3	2.8	1.9	2.8	1.9	2.1	0.39	1.8
	s.d.....		1.3	0.2	0.5	0.2	1.0	0.5	0.6	0.6	0.7	0.08	0.4

of arginine is in agreement with results on sweat (5) which contains relatively much more arginine than blood plasma (4). The general patterns of the free amino acids in spinal fluid and plasma, while showing no extreme variations, are not closely similar. The mechanism by which such differential concentrations are maintained is not clear. It is believed, however, that these values for amino acids in spinal fluid, together with concentrations found after feeding large amounts of individual amino acids, will throw some further light on the physiology of the secretion.

We have also carried out some analyses on the protein of spinal fluid. The material used was a mixture of coagulated protein obtained from the spinal fluids assayed for amino acids. The protein so obtained was hydrolyzed by the method of McMahan and Snell (7). The pattern of free

TABLE II

Free Amino Acid Content of Cerebrospinal Fluid from Ten Epileptic Subjects
Reported as micrograms of amino acid per cc. of cerebrospinal fluid.

Specimen No.	Age	Arginine	Histidine	Isoleucine	Leucine	Lysine	Phenylalanine	Threonine	Tyrosine	Valine	Methionine	Cystine
	<i>yrs.</i>											
1	31 ♂	6.1	2.3	0.50	1.4	1.9	1.6	2.4	1.9	2.1		
2	28 ♀	6.6	2.5	0.45	1.5	3.0	1.3	2.2	2.9	2.1		
3	26 ♀	6.4	1.4	1.0	1.7	2.8	1.1	1.4	1.6	1.1		
4	36 ♂	6.3	2.0	2.2	1.2	2.0	2.4	2.3	1.9	1.9		
5	34 ♂	6.9	1.9	0.94	1.0	1.7	2.1	3.5	2.2	1.5		
6	29 ♀	6.8	2.1	1.1	1.6	2.1	2.5	3.6	2.5	2.8		
7	26 ♂	5.5	2.0	0.70	1.4	2.9	2.6	3.2	2.6	1.8	0.29	2.5
8	45 ♂	6.7	1.6	0.60	1.8	4.4	3.1	3.0	3.2	2.7	0.47	2.0
9	57 ♂	8.2	1.4	0.55	1.3	3.1	2.0	3.9	1.8	2.1	0.45	2.4
10	27 ♂	6.6	1.5	0.75	1.5	2.9	1.5	3.8	2.0	2.5	0.47	1.5
Mean		6.6	1.8	0.87	1.4	2.6	2.0	2.9	2.2	2.0	0.42	2.1
S.D.		0.7	0.3	0.4	0.2	0.7	0.6	0.7	0.5	0.5	0.07	0.4

Recapitulation for all subjects

Combined mean*	6.0	1.7	0.98	1.4	2.8	1.9	2.8	2.0	2.1	0.40	1.8
S.D.	1.4	0.5	0.5	0.2	0.8	0.7	0.9	0.7	0.5	0.09	0.5

Comparison of two composite samples

Group A, old	5.0	1.7	0.68	1.2	2.4	1.8	2.7	2.4	1.6	0.36	1.2
“ B, young	2.6	0.70	0.41	0.81	1.4	0.80	1.3	1.1	1.1	0.20	0.60

* Twenty-six specimens.

amino acids in blood plasma has been shown to follow quite closely the pattern of the combined amino acids in the blood plasma proteins, suggesting some relationship. The free amino acids of spinal fluid do not follow closely the pattern of the amino acids in the spinal fluid protein, which suggests a lack of relationship. In spinal fluid (Table III) the ratio of the concentration of arginine to that of histidine is 3.5, while in the protein of spinal fluid

the ratio is 1.0. The ratio of the tyrosine value to the valine value in spinal fluid is 0.9, while in the spinal fluid protein the ratio is approximately 0.4.

The values for spinal fluid protein show a certain similarity with those for ox serum albumin (8) and would be reasonably consistent with the view that the spinal fluid protein is a combination of serum albumin with some pseudoglobulin and perhaps small amounts of other proteins. It is generally believed that the protein of spinal fluid consists largely of serum albumin. Dr. C. A. Johnson tested some of our specimens, using the precipitin reaction, for human serum albumin and confirmed this. He also showed, by use of the precipitin reaction, that small amounts of human pseudoglobulin were present.

TABLE III

Comparison of Free Amino Acids in Cerebrospinal Fluid with Those in Human Plasma and in Amino Acids of Cerebrospinal Fluid Protein

Amino acids	Free amino acids,* mean and s.d.		Ratio, Plasma Spinal fluid	Spinal fluid protein†
	Spinal fluid	Plasma		
Arginine.....	6.0 ± 1.4	23 ± 6	4	5.42
Histidine.....	1.7 ± 0.5	14 ± 2	8	5.16
Isoleucine.....	0.98 ± 0.5	16 ± 3	15	1.75
Leucine.....	1.4 ± 0.2	20 ± 3	15	6.22
Lysine.....	2.8 ± 0.8	29 ± 4	10	13.05
Phenylalanine.....	1.9 ± 0.7	14 ± 3	8	3.16
Threonine.....	2.8 ± 0.9	20 ± 4	7	4.06
Tyrosine.....	2.0 ± 0.7	15 ± 4	8	1.97
Valine.....	2.1 ± 0.5	28 ± 3	14	5.00

* Reported as micrograms of amino acid per cc. of cerebrospinal fluid and plasma.

† Reported as amino acid nitrogen in per cent of total nitrogen.

The possibility of the presence in spinal fluid of amino acids as "conjugates" or in peptide combination is recognized. The presence of such forms in plasma has been reported (9). The values of Harris (3) for glutamine in cerebrospinal fluid comprise about 69 per cent of the spinal fluid total α -amino nitrogen. Christensen *et al.* (2) report the glycine concentration of spinal fluid as being one-tenth and the alanine concentration one-third of the plasma levels. Gutman and Alexander (10) report plasma levels of 8.0 and 16.1 mg. per cent for glycine and alanine respectively. Our results on eleven individual amino acids show them to constitute approximately 18 per cent of the total α -amino nitrogen reported for cerebrospinal fluid (2). On adding the values for glutamine, glycine, alanine, and the values for the eleven individual amino acids, one gets a value of approximately 94 per cent of the total, using the average value (8.95 mg. per cent) for glutamine as

reported by Harris (3). We were not able to demonstrate the presence of free proline in spinal fluid. Our results with glutamic acid indicate that it is present in amounts not greater than 2 per cent of the glutamine value. On the basis of these findings it appears improbable that the presence of "conjugates" or other peptide combinations has a great effect, if any, on the determination of the individual amino acids in spinal fluid by the microbiological methods employed.

SUMMARY

A method for the microbiological determination of free amino acids in cerebrospinal fluid is described. Results are presented for eleven individual free amino acids in the spinal fluid of twenty-six subjects. Average values, in micrograms per cc. were as follows: arginine 6.0 ± 1.4 , histidine 1.7 ± 0.5 , isoleucine 0.98 ± 0.5 , leucine 1.4 ± 0.2 , lysine 2.8 ± 0.8 , phenylalanine 1.9 ± 0.7 , threonine 2.8 ± 0.9 , tyrosine 2.0 ± 0.7 , valine 2.1 ± 0.5 , methionine 0.4 ± 0.09 , and cystine 1.8 ± 0.5 . The values vary from about one-fourth to one-fifteenth of those for blood plasma.

No significant changes were noted in the free amino acids in the spinal fluids of epileptic or other patients studied.

An analysis of spinal fluid protein is reported.

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